Ryoko IMAICHI*: **Developmental anatomy of the shoot apex of leptosporangiate ferns I. Leaf ontogeny and shoot branching of *Dennstaedtia scabra*****

今市涼子*: 薄嚢シダ類の発生解剖学 I. コバノイシカグマの葉原基形成とシュートの分枝

(Pl. VIII–XI)

Not so many critical examinations have been done to clarify the mode of leaf ontogeny of the leptosporangiate ferns. The interpretations presented by several workers seem to belong to either of the following two views: (1) the leaf primordium is formed only from a single superficial cell of the apical meristem (Bower 1923, Bartoo 1930, Sifton 1944, etc.) and (2) the leaf primordium is originated in a group of cells of the apical meristem and is therefore comparable with that of the seed plant in origin (Steeves & Briggs 1958, Hagemann 1964, Hébant-Mauri 1975, etc.). Recently Imaichi (1982) favored the latter view based on study of *Hypolepis punctata*, though she suggested a different opinion that the difference between these two views mentioned above seems to be due to the difference in interpretation of the construction of the leaf primordium itself. The leaf primordium is considered to consist of only the leaf apical cell and its derivatives in the former view, but to include also the adjacent cells in the latter view. To reach a conclusion regarding this problem detailed investigation of the mode of leaf ontogeny of various leptosporangiate fern groups is required.

For the present study *Dennstaedtia scabra* (Wall.) Moore was selected. *Dennstaedtia* is known as a genus with buds on the petiolar base, the so-called petiolar buds, like several other dennstaedtioid genera (Troop & Mickel 1968). However, *D. scabra* distributed over East Asia and South East Asia has no such bud. Comparison of the leaf ontogeny of *D. scabra* with those of *Histiopteris incisa* and *Hypolepis punctata*, both of which have typical petiolar buds (cf.

* Faculty of Agriculture, Tamagawa University, Tamagawa-Gakuen 6-1-1, Machida-shi, Tokyo 194. 玉川大学 農学部.

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Imaichi 1980, 1982, 1983), should contribute to the elucidation of the phylogenetic origin of the petiolar bud.

Materials and methods Rhizome tips of *Dennstaedtia scabra* were collected in the environs of Owase City, Mie Prefecture. They were fixed with Craf I solution (Sass 1958) for 24 hr and embedded in paraffine, then sectioned longitudinally or transversely at a thickness of 6 μm. The longisections were cut to include the center of the apical meristem and the growing leaf primordium or the site of the next leaf primordium, and other longisections were cut in the frontal plane through the long axis of the ellipsoidal apical mound of the just-dichotomizing shoot tip. The sections were stained with a combination of Heidenhein's hematoxylin, safranin, and fast green.

Observations Gross morphology. The rhizome of *Dennstaedtia scabra* creeps on or just below the ground surface. Leaves (L) alternate in two rows on the upper side of the rhizome, which often branches into two shanks approximately equal, but in some cases somewhat unequal, in diameter (Fig. 1A, B). There is no close relation between the sites of leaf insertion and the rhizome dichotomy (Fig. 1A), although the leaf is sometimes borne on the bifurcated portion of the rhizome (Fig. 1B).

Organization of the shoot apex. The shoot apical meristem is rather convex, showing an organization typical for other leptosporangiate ferns (Pl. X-A). A tetrahedral apical cell(a) which cuts off its segments on three lateral sides (Pl. VIII-A) in regular, sometimes irregular, sequence (cf. Imaichi 1982) and many oblong prismatic cells occupy the surface, covering the subsurface group of smaller cells which differentiate into the pith (pt, Pl. X-A). The peripheral

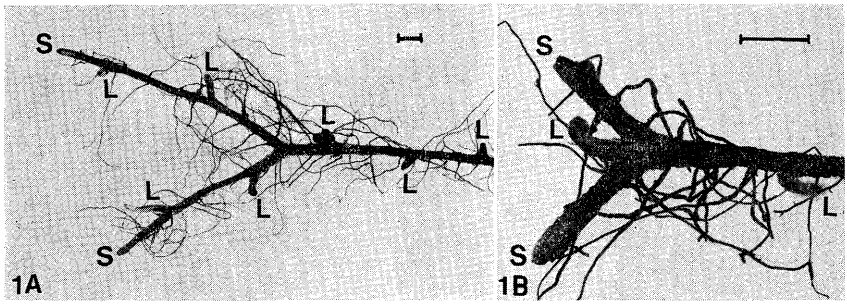

Fig. 1. Rhizome forking. A, Forking is independent of leaf insertion. B, Leaf arises from the fork of two shanks. L, Leaf; S, shoot tip. Scale, 1 cm.

region of the apical meristem also has a group of smaller cells which differentiate into the cortex(ct) and the procambium(pc). In some samples, the prominent apical cell is not recognized easily. This may be related to relatively frequent dichotomy of the rhizome tip as described later.

The cortex of *D. scabra* is not very broad, being about twenty cell layers thick, and the 'primary thickening meristem' is not apparent; it was described by Stevenson (1976) for *D. cicutaria* as a generalized zone of the cells which divide predominantly in periclinal plane.

Initiation and early development of the leaf primordium. The mode of initiation and very early development of the leaf primordium of *D. scabra* is very similar to that of *Hypolepis punctata* (Imaichi 1982). The first indication of leaf initiation is an appearance of an enlarged cell (asterisk) with a large, lightly stained nucleus on the flank of the apical meristem (Pl. VIII-E). This enlarged cell cuts off the leaf apical cell lenticular in shape(1a) through two to four subsequent cell divisions in the oblique plane (Pl. VIII-F). The segmentation patterns to establish the leaf apical cell in this enlarged cell are various in transectional view (Pl. VIII-B, C, D).

The leaf apical cell with its segments, that is, the group of derivatives of the enlarged cell mentioned above ('leaf apical cell complex', Imaichi 1982) takes a various positions, e. g. at the center (Pl. VIII-B) or on the edge (Pl. VIII-A), of a cell packet which have been derived from a segment of the shoot apical cell. Thus, as in *Hypolepis punctata* (Imaichi 1982), the correlation between leaf initiation and the apical segmentation pattern of the shoot is not very clear. This interpretation seems to be also suggested by the following fact. In some samples of dichotomizing shoot tips of *D. scabra*, the leaf primordium begins to develop from one of the superficial cells lying between already established two apical cells (Pl. XI-C). Here it is not clear which one of these two apical cells produced the superficial cell initiating the leaf primordium as a result of respective apical segmentation. It appears that the superficial cell is independent of both of the apical cells.

As the leaf develops, the leaf apical cell continues to divide forming a well-discernible packet of cells, 'leaf apical cell complex' (the part shown by arrows in Pl. VIII-F, G, IX-A, B, C). The developmental stage of the given leaf primordium is represented by the number of segments produced in the leaf apical cell complex. Up to the 3-segment stage, the cells surrounding the leaf apical cell complex

itself hardly divide, but elongate to construct the elevated mound of the leaf primordium together with the leaf apical cell complex itself (Pl. VIII-F). After this stage, in turn, such cells begin to divide frequently in anticlinal and even in periclinal planes, contributing to broadening of the basal part of the young leaf primordium (Pl. VIII-G, IX-A, B). Thus the leaf primordium of *D. scabra* is composed of two groups of cells, the leaf apical cell complex and the basal cells surrounding it.

The surface cells of the abaxial basal part of the leaf primordium remain fairly elongate shape until the 3- or 4-segment stage (Pl. VIII-F, IX-A), presenting images very similar to those found with *Histiopteris incisa* (Figs. 15, 17 in Imaichi 1980) and *Hypolepis punctata* (Figs. 7, 17 in Imaichi 1982). After the 5-segment stage, those surface cells of *D. scabra* become short and increase in number through frequent cell divisions in the periclinal plane (Pl. IX-B, C). In the same stage of *Histiopteris incisa* and *Hypolepis punctata*, however, the surface cells remain elongate and differentiate later into the first petiolar bud (see Fig. 18 in Imaichi 1980, and Fig. 19 in Imaichi 1982).

At the 7-segment stage, the outline of the leaf apical cell complex becomes obscure, though in some cases the probable outline is discernible from the cell arrangement and cell lineage (two arrows in Pl. IX-D). After this stage, the outline is hardly recognized and then it becomes impossible to judge exactly what extent the basal cells contribute to further development of the leaf primordium (Pl. X-A). As the group of cells (M, cf. Imaichi 1982) between the leaf primordium and the shoot apical meristem increase in number and size, the leaf primordium becomes further separated from the shoot apical meirstem (Pl. X-A, B, C). The typical intercalary meristem, found in the region below the petiolar bud in *Hypolepis punctata* (Imaichi 1983), can not be found on either the adaxial or the abaxial basal region of the leaf primordium of *D. scabra*. The activity of the basal cells for construction of the leaf base may be less than thatof *Hypolepis punctata*.

Rhizome dichotomy. Transection of the shoot tip at the earliest stage of dichotomy shows that an apical cell becomes to disappear, and a cell packet square in outline (indicated by four arrowheads), instead of an apical cell, occupies the central region of the apical meristem (Pl. XI-A). This cell packet does not include the probable apical cell triangular in shape and the rest of the apical meristem also does not seem to have such a triangular cell. The longi-

section of other sample shows that the probable apical cells (a in Pl. XI-B) almost equal in size are in contact with each side of a square cell packet (indicated by two arrowheads, Pl. XI-B). These findings seem to indicate that, when the shoot apex is bifurcated, the old apical cell stops functioning and two new apical cells are formed later. The bifurcation of the shoot tip does not seem to be caused by equal division of the apical cell into two daughter cells, each of which functions as a new apical cell.

After both apical cells are established on the apical meristem they move apart from each other during further development, each of which organizes its own apical meristem (Pl. XI-C, D). As the result, the old apical meristem is divided into two new apical meristems approximately equal in size. Formation of somewhat unequal branches, strong and weak (Fig. 1A), seems to be caused by the difference in activities of the newly formed apical meristems.

When a leaf is formed in either of the two newly established apical meristems, or in the mid region between them, structures found in *D. scabra* are very similar to those of *H. punctata* in which the bud meristem initiated in the basal region of the leaf primordium has already developed to a size nearly equal to the shoot apical meristem (compare Pl. XI-E, F with Fig. 4 in Imaichi 1983). Both examples of *D. scabra* and *H. punctata* are alike in a fairly developed stage, but probably differ in their initial stages. The bud merisem of *H. punctata* is considerably smaller than the shoot apical meristem at the earliest stage of development, and establishment of the apical cell in the bud meristem is somewhat delayed. In *D. scabra*, however, both shoot apical meristems were equal in size from the beginning, and division of the shoot apical meristem occurred independently of leaf initiation.

Discussion After critical examination of various fern groups, Bierhorst (1977) concluded that the origin of the leaf primordium does not initially involve the cells in the vicinity of the leaf mother cell (the cell which cuts off the leaf apical cell) and those cells are involved in later changes. However, with *Dicksonia squarrosa*, Hébant-Mauri (1975) found that a small group of 'activated' cells can be recognized as the first indication of future leaf initiation, and the leaf initial cell (the cell which cuts off the leaf apical cell) and the neighboring cells together construct the most typical stage of leaf initiation. The mode of leaf initiation in *Hypolepis punctata* and *Histiopteris incisa* (Imaichi 1980, 1982) is the same as that in *D. squarrosa*. The present study has shown that leaves

of *Dennstaedtia scabra* also develop in the same manner. When very young, the leaf primordium consists of not only the leaf apical cell complex, derivatives of Hébant-Mauri's leaf initial cell or of Bierhorst's leaf mother cell, but also of neighboring basal cells. This seems to favor the second view of leaf initiation mentioned above, i. e., the leaf primordium is initiated in a group of cells in the apical meristem.

The so-called petiolar buds of *Histiopteris incisa* and *Hypolepis punctata* were considered to be phyllogeneous in the sense that they originate in cells located in the basal region of the elevated mound of the young leaf primordium, and then the thus-formed bud meristems are carried up higher due to intercalary growth at the leaf base below the insertion of the bud (Imaichi 1980, 1982, 1983). From the phylogenetic point of view, however, this information from both species is insufficient for concluding whether the leaves of both species have the ability to initiate the bud, or the leaf gains this ability as a result of 'congenital fusion' with the lateral shoot.

This study showed that even in the case of *D. scabra* with no petiolar bud, actively dividing basal cells surrounding the leaf apical cell complex contribute to the leaf base broadening, as in *Histiopteris incisa* and *Hypolepis punctata*. Some of these basal cells differentiate into the bud meristem in the latter both species, but not in *D. scabra* where they differentiate into ordinary cells constructing the leaf base. This may suggests that the leaf base has the ability to initiate the bud in some groups of ferns with buds in their leaf bases.

The typical intercalary meristem shown in *H. punctata* (Imaichi 1983) was not found here. Then the ratio of basal cells surrounding the leaf apical complex to a well-developed leaf primordium in *D. scabra* is presumed to be smaller than that of *H. punctata*. Thus the difference between these both species became apparent at relatively later stage of leaf development. The available information on the leaf ontogeny is not yet sufficient to evaluate the significance of such difference or to interpret the phylogenetic identity of the petiolar shoot. The modes of leaf ontogeny of several other fern groups need to be elucidated also.

Few critical investigations have been done to elucidate the mode of dichotomy of the shoot apex with a single apical cell. Two modes have been thus far reported: (1) the apical cell divides equally into two daughter cells, each of which functions as the apical cell of a branch (Cross 1931, Bierhorst 1977) and (2) the old apical cell ceases its growth and two new apical cells are established

later (Gottlieb & Steeves 1961, Hagemann & Schulz 1978, Mueller 1982). The mode of bifurcation clarified here in *D. scabra* is very similar to that of *Lygodium* described by Mueller (1982), which appears to support the latter view. The little difference in length or diameter between two shanks, strong and weak, of *D. scabra* is not caused by the so-called lateral branching but by a difference in activity between the new apical mristems after the bifurcation of the shoot tip. According to Hagemann & Schulz (1978) and Mueller (1982), the dichotomizing apical meristem widens first and then divides into two, on each of which a new apical cell is formed. In comparison to this, new apical cells of *D. scabra* become perceptible earlier, that is, before the dividing of the apical meristem. The two mounds of the apical istemmer seem to be formed somewhat later as a result of the activities of the newly formed apical cells.

In some samples of *D. scabra*, where the weaker shank of unequal dichotomy has the developing leaf, the relationship among the both shanks and the leaf is very similar in appearance as well as in vasculature to the relationship among the shoot, leaf and the petiolar bud of *Hypolepis punctata* (Figs. II-2, -8 in Mitsuta 1976). However, the present examination has shown that these both cases are different in developmental mode. Leaf initiation of *D. scabra* occurs independently of rhizome branching and the leaf never initiates the bud, while in *H. punctata* it is the leaf primordium that initiates the bud.

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Literature cited

Bartoo, D. R. 1930. Bot. Gaz. **89**: 137–152. Bierhorst, D. W. 1977. Amer. J. Bot. **64**: 125–152. Bower, F. O. 1923. The ferns, vol. 1. Cambridge Univ. Press, Cambridge. Cross, G. L. 1931. Bot. Gaz. **91**: 65–76. Gottlieb, J. E. & T. A. Steeves 1961. Phytomorph. **11**: 230–242. Hagemann, W. 1964. Beitr. Biol. Pfl. **40**: 27–64. —— & U. Schulz 1978. Bot. Jahrb. Syst. **99**: 380–399. Hébant-Mauri, R. 1975. Can. J. Bot. **53**: 764–772. Imaichi, R. 1980. Bot. Mag. Tokyo **93**: 25–38. —— 1982. Bot. Mag. Tokyo **95**: 435–453. —— 1983. Bot. Mag. Tokyo **96**: 159–170. Mitsuta, S. 1976. Acta Phytotax. Geobot. **27**: 149–156 (in Japanese). Mueller, R. J. 1982. Bot. Gaz. **143**: 319–330. Sass, J. E. 1958. Botanical microtechnique. 3rd. ed. Iowa State Univ. Press, Ames.

Sifton, H. B. 1944. New Phytol. **43**: 87–129. Stevenson, D. Wm. 1976. Amer. J. Bot. **63**: 673–678. Steeves, T. A. & W. R. Briggs. 1958. Phytomorph. **8**: 60–72. Troop, J. E. & J. T. Mickel 1968. Amer. Fern J. **58**: 64–70.

Explanation of Plates VIII–XI

Plate VIII. Leaf initiation on the apical meristem. A–D are photomicrographs and A′–D′ are their diagrammatic illustrations. A–D and G, transections; E and F, longisections of the shoot tip. Numerals indicate the sequence of segmentation of the shoot apical cell or the leaf apical cell. A, A′, Dotted area indicates the leaf apical cell complex (see text). B, B′, C and D, Various segmentation patterns found in the leaf apical cell complex. Arrow indicates the direction of the shoot apical meristem. E, An enlarged prismatic cell (asterisk) shows the initiation of a leaf apical cell complex. F, Leaf primordium at the 2-segment stage. Arrows indicate the boundary of the leaf apical cell complex. G, Leaf primordium more developed than that of F. Arrows indicate the boundary of the leaf apical cell complex. a, shoot apical cell; a′, site of the shoot apical cell not included in this section; la, leaf apical cell. Scale, 100 μm.

Plate IX. Longisections of the leaf primordia at several developmental stages. A–C and D are photomicrographs and A′–C′ are their diagrammatic illustrations. Arrows indicate the boundary of the leaf apical cell complex. Numerals indicate the sequence of segmentation of the leaf apical cell. A, A′, 4-segment stage. B, B′, 5-segment stage. C, C′, 6-segment stage. D, Most developed leaf primordium among the figures in this plate. a′, site of the shoot apical cell not included in this section; la, leaf apical cell. Scale, 100 μm.

Plate X. Longisections of fairly developed leaf primordia. Developmental stage: A, youngest; B, middle; C, oldest. a, apical cell; ct, cortex; I, the newest leaf primordium; L, leaf primordium; M, intervening mound; pc, procambium; pt, pith; S, shoot apical meristem. Scale, 100 μm.

Plate XI Shoot dichotomy. A is the transection and B–F are longisections of the dichotomizing shoot tip. A, Arrowheads indicate the boundary of the cell packet on the central region of the shoot apical meristem. B, Two arrowheads indicate the boundary of the cell packet square in outline, on both sides of which two probable apical cells are already formed. C, Two arrows indicate the boundary of the leaf apical cell complex developing in the mid region

between both apical cells. D, Each apical cell has organized its own apical meristem. E, Leaf primordium has been initiated in one of the two newly established apical meristems. F, Leaf primordium is developed in almost the mid region of both apcial mersitems. a, apical cell; L, leaf primordium; S, shoot apical meristem. Scale, 100 μm.

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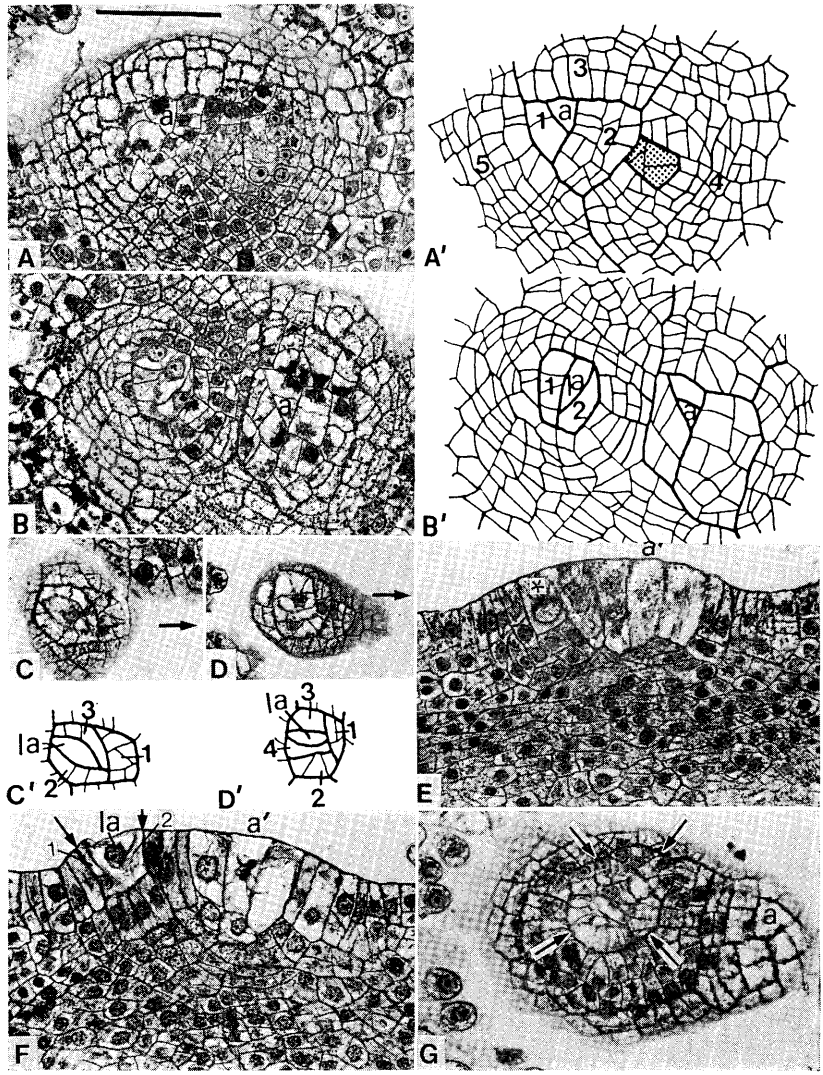
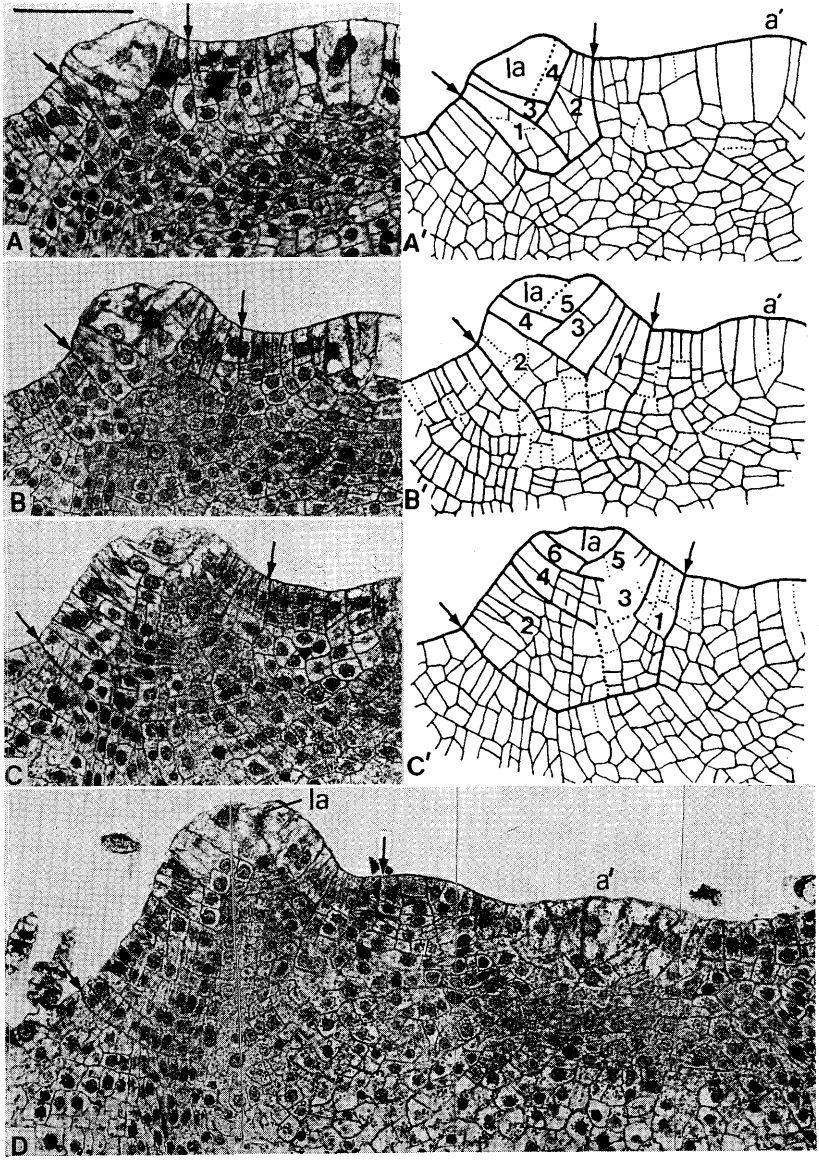
コバノイシカグマ属はイワヒメワラビ属,ユノミネシダ属などと同様,葉柄基部にいわゆる葉柄芽をもつグループとして知られているが,本邦産のコバノイシカグマは葉柄芽をもたず分枝は根茎の二又分枝による。イワヒメワラビ,ユノミネシダと同様,コバノイシカグマの葉原基は茎頂の表層細胞数個が起原となって発生を開始する。それらのうち中央の1細胞がレンズ型の葉原基頂端細胞を切り出し葉原基の上部を作り,一方それをとりまくまわりの細胞群は葉原基の基部を作る。イワヒメワラビ,ユノミネシダではこの基部細胞群のうち背軸側あるいは向軸側に位置する表層細胞群がもっぱら垂層分裂を行い芽を分化するが,コバノイシカグマではそのような特別な細胞群はみられず,すべての基部細胞群が並層分裂をさかんに行い芽を分化する事はない。以上から葉原基の初期発生の様式と葉原基構成という点ではコバノイシカグマとイワヒメワラビ及びユノミネシダとの間には差は見られない事がわかり,イワヒメワラビ,ユノミネシダの葉原基発生様式には葉柄芽をもつための特殊化はおこっていないと言える。

根茎の二又分枝では,まず茎頂の頂端細胞が識別できなくなる。その後,もとの頂端細胞の位置をはさむように茎頂に新しい頂端細胞が2個形成されて,これらがそれぞれの頂端分裂組織を作ることになる。

□Santesson, Rolf: **The lichens of Sweden and Norway.** 333 pp. 1984. Swedish Museum of Natural History, Stockholm. スエーデン及びノルウェー産地衣類のカタログである。著者は各種の文献や学名の取扱いに精通していることでは,現在の世界の地衣学界随一であり,この点からも本書の出現が待たれていた。最近20年ぐらいの地衣学の進展は目覚ましく,新属や新種の発見,学名の組換えなど枚挙にいとまもなく,標本整理のための参考文献が必要であるとの見地から本書を企画したとしている。そのためもあって,本書はむしろチェックリストと云う方が妥当かもしれない。各属の最後にシノニムの一覧表があったり,生態,分布の簡単なノート,とくに重要な文献があげられていたり,便利な点が多い。定価は不明だが,P.O. Box 50007, S-10405 Stockholm にある上記の博物館へ申込めば入手できると思う。 (黒川 道)



R. IMAICHI: Shoot apex of leptosporangiate ferns



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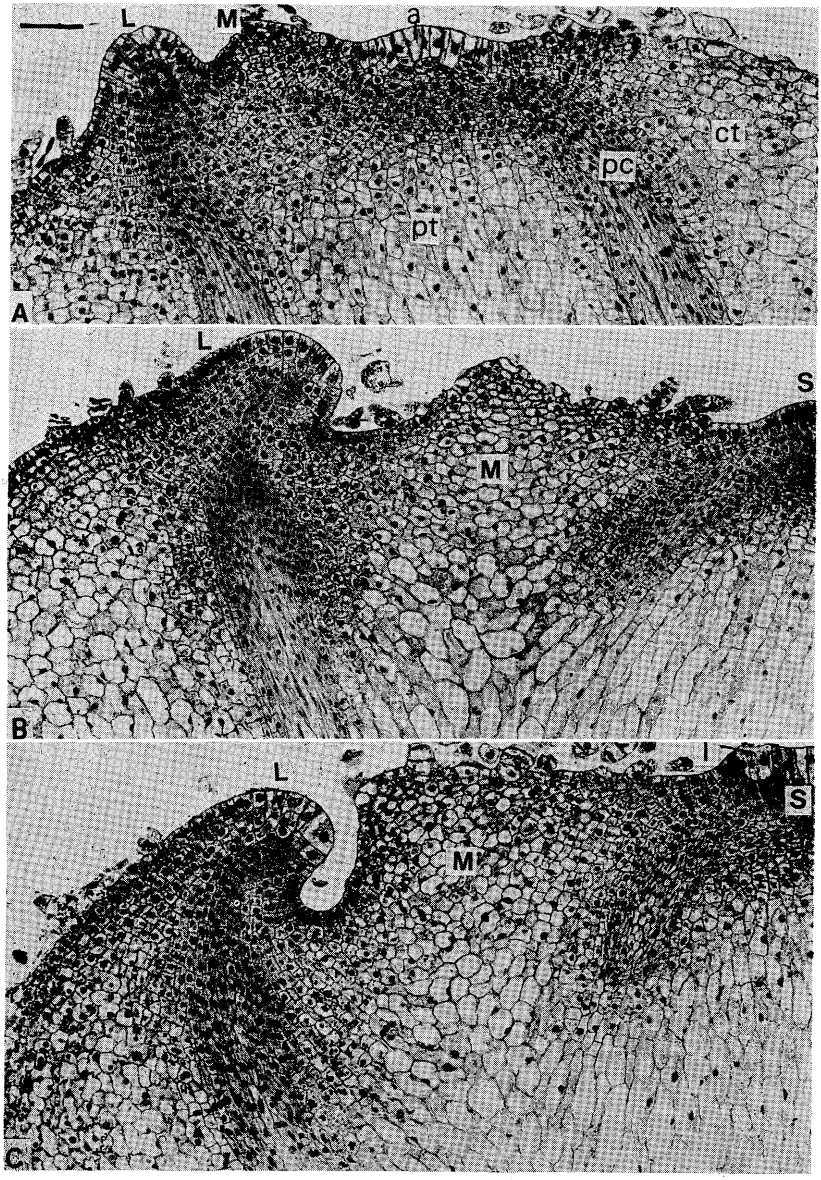

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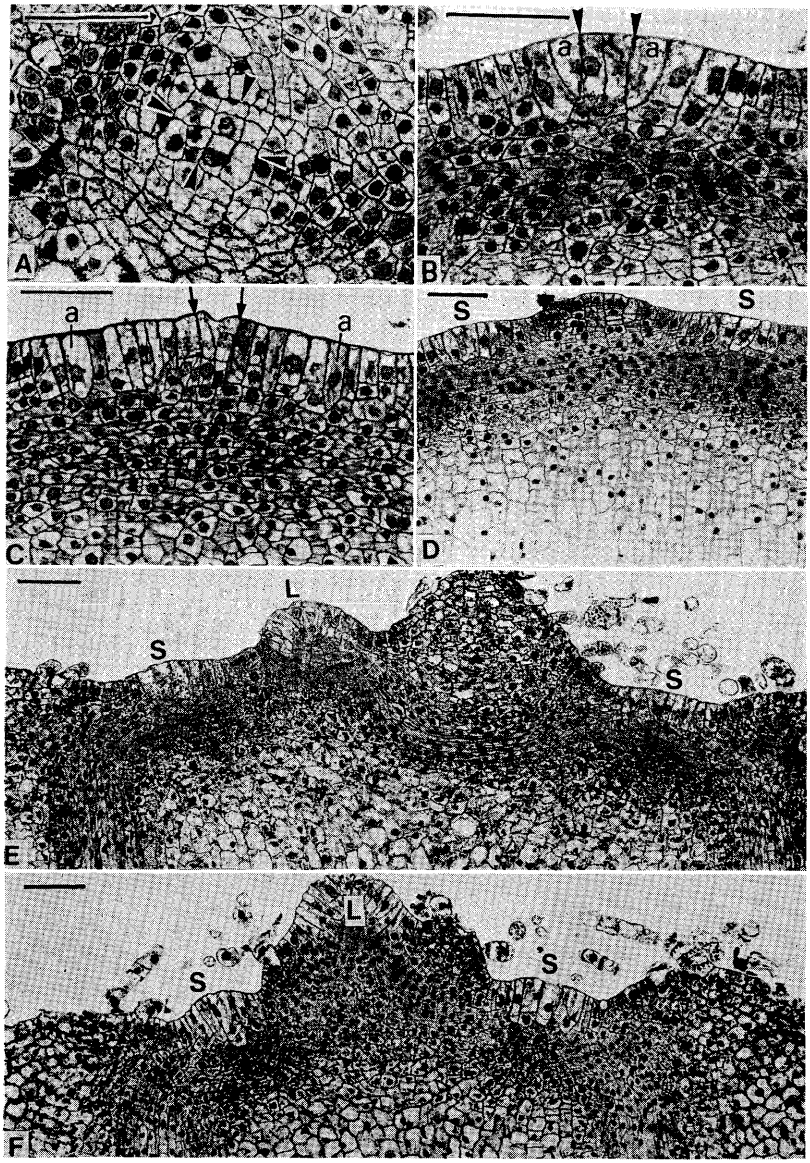

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